

VIABILITY EXTENSION OF STORED CHILLI SEED BY SOAKING AND DRYING*

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Large and small seeds of 2, 9 and 12 month old seeds of Chilli Co. 1 were soaked in Sodium phosphate (di-basic) solution at 10⁻⁴M for three hours and dried back to their original weight and stored for one year with and without artificial ageing. Hydration and dehydration with phosphate solution significantly slowed down the rate of deterioration of both large and small seeds of 9 and 12 month old seed stored without accelerated ageing; this treatment proved beneficial for both large and small sized seeds of 2, 9 and 15 month old seeds acceleratedly aged and stored.

The general decline in the viability and vigour of carry over seeds could be the effect of ageing accelerated by the critical fluctuations in RH and/or temperature of the storage environment besides the activity of the seed-borne pathogens and its concomitant effect on the catabolic changes promoting irreversible physiological, biochemical and membrane deteriorations. The storage conditions that maintain seed viability are those which slow down the respiration and other metabolic processes without injuring the embryo. The success achieved in maintaining seed viability in conventional, cold or dry storage is explainable if viewed as physical attempt to minimise chemical reactions going on within the seed and possibly within associated biotic agents.

Sivanayagam and Manokaran (1973) studied the effect of imbibition and drying cycles with water for 12-48 h

on the life span of chilli seeds and found such pretreatment of seeds for 12-24 h improved their life-span.

Basu *et al.* (1975) reported that soaking 3 to 10 month old seeds of chilli CV Suryamukhi, in water or solution of di sodium hydrogen phosphate (10⁻⁴M) and tannic acid (10⁻⁴M—10⁻⁶M) for three hours followed by immediate drying back to original weight extended their subsequent storage life under room temperature and 100 per cent relative humidity for one to five months.

An attempt was therefore, made to study the effect of pre-soaking chilli seeds in sodium phosphate (di-basic) solution and drying on their shelf life under natural and artificial ageing conditions and the beneficial effect of extended shelf life conferred by this treatment is discussed in the light of current theories of ageing

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MATERIALS AND METHODS

Three seed lots of chilli Co. 1 differing age namely, L1 (2month old), L2 (9 month old) and L3 (12 month old) were cleaned and size graded with BSS. 3 (aperture width 2 mm). The seeds retained by this sieve constituted the large seeds (G1)-100 seed weight 521 mg and the seeds that passed through this sieve constituted the small seeds (G2)-100 seed weight 425mg. The seeds of the respective size grades in each of the seed lots were divided into two equal portions and one portion of seeds was soaked in double the volume of sodium phosphate (di basic) for three hours and quickly dried back to their original weight (Basu *et al.*, 1975) first under electric fan and then sunlight (T1). The other unsoaked portion of seeds served as control (T0) which was also dried along with the T1 seeds.

a. Storage of seeds after accelerated ageing

The treated and untreated seeds of each lot differing in age were again divided into sub lots and each sub-lot was taken in perforated paper bags and subjected to accelerated ageing at $40 \pm 1^\circ\text{C}$ temperature and $98 \pm 2\%$ RH for 12 days in an ageing cabinet. Then the seeds removed and quickly dried under ambient temperature and RH. They were periodically evaluated at trimonthly intervals (P0, P3, P6, P9, P12) for the germinability and vigour potentials by the standard germination test (Anon, 1976) and measurement of root and shoot length, assessment of drymatter production per normal seedling at final count for germinability and estimation of vigour

index (Abdul-Baki and Anderson 1973) respectively.

Germination test was carried out with 4x100 seeds using the paper-towel medium. The vigour index was determined by multiplying the percent germination at final count with mean total seedling length in cm and expressed as whole number.

b. Storage of seeds with out accelerated ageing

The treated and untreated seeds of the three lots were stored without accelerated ageing in paper bags under ambient temperature and RH. The relative storability of these seeds were evaluated initially (P0) and at third (P3), sixth (P6), ninth (P9) and twelfth (P12) month after storage as detailed above.

RESULTS AND DISCUSSION

a. Storage of seeds after accelerated ageing (Table 1)

Standard Germination

The treated seeds recorded high germination percentage of 54 compared with control (32%).

The germination percentage of treated seeds of L1, L2 and L3 differed significantly from control seeds belonging to the respective lots; while the treated seeds of L1, L2, L3 recorded respectively 80, 49 and 33% germination compared with 65, 17 and 14% recorded by the control seeds of the respective lots. The germination percentages of treated seeds of G1 and G2 sizes were respectively 60 and 47, compared with 32 and 31 recorded by the control seeds of the respective

Table 1: Effect of hydration and dehydration on the germinability and Vigour of Co. 1 Chilli seeds stored after accelerated ageing

Treatment	Period					Grade			Seed Lot			Mean	T	TxG	TxL
	P ₀	P ₁	P ₂	P ₃	P ₄	G ₁	G ₂	L ₁	L ₂	L ₃					
Germination %															
T ₀	43 (40.3)	43 (41.7)	34 (34.5)	25 (82.0)	15 (21.0)	32 (33.5)	31 (32.8)	65 (54.7)	17 (23.5)	14 (21.3)	32 (33.2)	CD 1.68**	2.38**	2.90**	
T ₁	70 (60.1)	67 (56.5)	57 (50.1)	46 (42.7)	28 (31.1)	60 (52.0)	47 (44.2)	80 (65.8)	49 (44.0)	33 (34.5)	54 (48.0)				
Root length of seedling (cm)															
T ₀	7.21	6.93	6.59	6.00	5.56	6.88	6.06	7.55	6.24	5.59	6.46		—	NS	
T ₁	8.47	7.79	7.22	6.86	6.41	7.98	6.72	8.15	7.76	6.14	7.35	CD 0.27**	—	0.47**	
Shoot length of length seedling (cm)															
T ₀	7.48	7.23	6.43	6.19	5.36	6.70	6.38	8.21	6.25	5.16	6.54		—		
T ₁	8.08	7.87	7.22	7.25	6.32	7.31	7.39	8.97	7.18	5.89	7.35	CD 0.21**	0.10 0.29**	—	
Dry matter production per seedling (mg)															
T ₀	2.08	1.98	1.83	1.59	1.30	1.91	1.61	1.96	1.72	1.60	1.76		—	—	
T ₁	2.32	2.11	1.97	1.80	1.48	2.11	1.76	2.01	1.92	1.79	1.93	CD 0.14*	NS	—	
Vigour index															
T ₀	675	664	498	349	198	504	445	1053	217	154	475		—	—	
T ₁	1204	1087	882	693	394	982	723	1384	754	418	852	CD 102**	NS	—	

Table 2: Effect of hydration and dehydration on the germinability and Vigour of Co. 1 Chilli seeds stored without accelerated ageing

Treatment	Period				Grade			Seed Lot			Mean	T	T x G	T x L
	P ₀	P ₁	P ₂	P ₃	G ₁	G ₂	G ₃	L ₁	L ₂	L ₃				
Germination %														
T ₀	88 (74.1)	85 (68.7)	78 (64.1)	69 (58.1)	59 (51.2)	74 (60.7)	78 (65.8)	92 (72.6)	71 (58.1)	65 (54.5)	76 (63.2)	CD 2.09**	1.09**	5.01**
T ₁	96 (82.3)	93 (77.7)	87 (72.0)	82 (65.3)	73 (60.2)	89 (72.9)	84 (70.5)	94 (77.6)	86 (71.6)	80 (65.9)	86 (71.7)			
Root length of seedling (cm)														
T ₀	7.80	7.58	7.26	6.46	5.95	7.30	6.72	7.63	6.17	7.25	7.01	NS	—	—
T ₁	8.20	7.90	7.88	7.00	6.50	7.95	7.03	8.08	6.90	7.50	7.49	CD 0.19**	—	—
Shoot length of seedling (cm)														
T ₀	9.42	9.08	8.58	8.05	7.67	8.79	8.31	9.67	7.68	8.32	8.55	NS	—	—
T ₁	9.16	8.55	8.38	7.89	7.20	8.42	8.04	9.15	7.90	7.64	8.23	CD 0.21**	—	0.36*
Dry matter production per seedling (cm)														
T ₀	2.22	2.10	2.04	1.98	1.83	2.22	1.84	2.04	1.91	2.15	2.03	NS		
T ₁	2.26	2.14	2.02	2.00	1.86	2.20	1.91	2.02	1.98	2.16	2.05	NS		
Vigour index														
T ₀	1528	1421	1247	1017	836	1209	1211	1593	1003	1034	1210	—	—	—
T ₁	1667	1537	1389	1246	1024	1447	1297	1618	1268	1231	1372	CD 78**	—	135**

sizes. The treated seeds of G_1 and G_2 , however, differed significantly in their percentage of germination.

Root and shoot length of seedling:

Treated seeds produced 7.35 cm long root compared with 6.46 cm produced by the seedling from the control seed. Seedlings from the treated seeds of L_1 , L_2 and L_3 differed significantly in their root length recording 8.15, 7.76 and 6.14 cm. Treated seed caused production of longer shoots (7.35 cm). Seedlings from the treated seeds of G_1 and G_2 size recorded shoot length of 7.31 and 7.39 cm respectively.

Dry matter production of seedling:

The seedling of treated seed produced high dry matter. (1.93 mg) compared with 1.76 mg (control).

Vigour index

The seedling from the treated seed recorded higher vigour index value of 852 compared with 475 recorded by the seedling from the control seed.

b Storage of seeds without accelerated ageing (Table 2)

Standard germination

The treated seeds recorded significantly high germination percentage of 86. The germination percentage of treated and control seeds of L_1 were on a par; the differences among the germination percentages of treated and control seeds of L_2 and L_3 were highly significant. The treated seeds of L_1 , L_2 and L_3 recorded 94, 86 and 80% germination, while the control seeds of the respective lots registered 92, 71 and 65 % germination.

The differences among germination percentage were significant due to the treatment in respect to seeds of both G_1 and G_2 sizes. The control seeds of G_1 and G_2 sizes differed significantly in their germinability. The treated seeds of G_1 and G_2 sizes were on a par, recording 89 and 84 % germination respectively.

Root and shoot length of seedling

The treated seed was highly superior in producing seedling with a root length of 7.49 cm. However, the control seed produced seedling with significantly long shoot measuring 8.55 cm compared with the treated seed (8.23 cm). The shoot lengths of seedlings from the treated and control seeds of L_1 and L_2 differed significantly. Untreated seeds of L_1 , L_2 and L_3 produced 9.67, 7.68 and 8.32 cm long shoots compared to 9.15, 7.00 and 7.64 cm produced by the seedlings from the seeds.

Dry matter production per seedling

The treatment did not influence the dry matter production of seedling.

Vigour index:

Seedlings from the treated seeds were superior with a vigour index value of 1372 compared to the seedlings from the control (1210). The vigour indices of seedlings from the treated and control seeds of L_1 were on a par; the difference among the vigour indices of seedlings from the seeds of L_2 and L_3 were highly significant. Seedlings from the treated seeds of L_1 , L_2 and L_3 recorded vigour index values of 1618, 1268 and 1231 respectively compared

with the seedlings from the control seeds registering 1593, 1003, and 1034 respectively.

Hydration and dehydration of Co. 1 chilli seeds with phosphate solution improved their immediate germinability as well as their shelf life when kept in open storage with and without accelerated ageing.

The immediate improvement in germinability following hydration and dihydration treatment is not due to the germination advancement, leaching of toxic materials, rapid imbibition of water or anti fungal effect but may be due to the reduction in lipid peroxidation (Basu 1976, Rudrapal and Basu 1979) resulting in the maintenance of normal structure of membranes. Membrane with a normal structure are antioxygenic in function with more resistance to lipid peroxidation and various types of molecular damage but not necessarily immune (Pryor 1971). The beneficial effect on seed longevity conferred by this treatment was in agreement with that reported in Sugar beet (Basu and Dhar, 1979), brinjal and onion (Basu *et al.*, 1975), chilli (Basu *et al.*, 1975; Sivanayagam and Manokaran, 1973), lettuce (Basu *et al.*, 1979), carrot (Basu *et al.*, 1975; Savino *et al.*, 1979) and pea (Savino *et al.*, 1979).

A free radical is any chemical species with a single unpaired electron (odd number of electrons) in an outer orbital. In biological systems, oxygen is so reactive that it is able to attack most organic materials spontaneously, although fortunately only slowly in auto-oxidative process and makes it potentially hazardous to the organisms

(Pryor, 1971). A number of biological oxidations both enzymatic and spontaneous, generate the super-oxide radical (O_2^-) which is cytotoxic and in turn can react with H_2O_2 to produce singlet-oxygen and the hydroxy radical ($OH\cdot$) i.e. highly potent oxidants (Fridovich, 1976). The membrane systems of the cell including those of organelles such as mitochondria, lysosomes and endoplasmic reticulum are the critical sites of oxidative damage when disrupted. Changes in the ability of the membranes to allow the diffusion of selected biochemical species would very critically affect the well being of the cell. Oxidation of the membranes will also lead to the disruption of the vital energy producing systems occurring either in close proximity or bound to the membranes (Pryor, 1971). In lipid auto-oxidation, the poly unsaturated fatty acids (PUFA) of membrane phospholipids react with free radicals of oxygen in a chain reaction, and one of the end products of this destructive process, malonaldehyde reacts with primary amino groups of macro-molecules forming irreversible iminopropene covalent linkages causing deterioration of membranes of lysosomes, mitochondria and microsomal fractions (Tappel, 1970; 1975; 1978). Deteriorative processes affecting the pre-existing systems were reported to occur in mature dry seeds due to the operation of genetic factor as well changes in the essential segments of the DNA can lead to impaired transcription and translation at the site of mitotic apparatuses and concomitantly lead to loss of viability (Abdalla and Roberts, 1968; Villiers, 1975; Bray and Dasgupta, 1976; Bewley and Black 1982). According to Roberts and Osborne (1973) and

Ching (1973) destructive changes in enzymes, nucleic acids mitochondria, ribosomes etc., may disrupt the cellular metabolism such as turn-over type maintenance, protein synthesis, glycolysis, fatty acid oxidation, solute ion transport, cytoplasmic streaming, respiration, building up of ATP content for the various synthetic processes and providing enough substrates for respiration and protein synthesis during the very early phase of germination eventually leading to loss of vigour and viability.

Though senescence is a part of cell's genetic programme (Marx, 1974) accumulation of environmental insults such as prevalence of high temperature and relative humidity of the storage environment, nutrient deficiency, radiation, pollutants etc., also (Rana and Munkres, 1978) will be particularly serious for the seeds (Marx, 1974; Taylor *et al.*, 1976)

Berjak and Villiers (1972) and Villiers (1973); are of the opinion that the free-radical damage accumulated during dry storage will cause the macromolecular peroxidation and the consequent extensive membrane damage and lysis of constituents of the embryonic cells due to the rupture of the lysosomes and the consequent spilling of the destructive hydrolytic enzymes during the imbibition phase, when seed becomes hydrated; if the repair mechanism becomes inoperative the seed loses its viability. Soaking seeds in water for an optimum period found effective in extending their viability could therefore, be attributed to (i) the fluidity or its reciprocal microviscosity of the lipid bilayer of cellular membranes, an important determinant

of the activity of membrane bound enzymes and other membrane functions (Vessey and Zakim, 1974) and (ii) the operation of cytosolic synthetic or repair mechanisms which might help to repair the membranes of cell and cellular organelles that had not been damaged to extensively by lipid auto-oxidation (Berjak and Villiers, 1972; Morohasi and Bewley, 1980; Wood and Powell, 1983). Savino *et al.*, (1979) speculated that some factor or factors activated during soaking and retained on dehydration could have affected ageing and helped the maintenance of viability and vigour of seeds in storage. The inorganic salt such as sodium phosphate applied through water might have stabilised the repaired membranes (Miller *et al.*, 1971).

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